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A Novel Bucco-Vaginal Controlled Release Drug Delivery System of Miconazole Nitrate for Candidiasis-Design and Evaluation.

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SUMMARY. **A variety of approaches have been studied in the past to overcome the problems encountered with the delivery of antifungal, for effective treating of oral and vaginal candidiasis. In this study, a novel mucoadhesive tablets with pH-independent drug release characteristic was prepared by chitosan and carbopol® 71G interpolymer complex (IPC) claims for multipurpose use. Precipitation method is employed for preparation of IPC followed by characterization with Fourier transform infrared spectroscopy (FT-IR) and Differential scanning calorimeter (DSC). Bucco-Vaginal Miconazole nitrate (MN) compacts were prepared by direct compression using IPC. The formulations were tested for physicomechanical properties, in vitro drug release (buccal and vaginal pH), swelling studies and mucoadhesion strength. The dissolution of MN from all the prepared tablets into the phosphate buffer (pH 6.8) and simulated vaginal fluid pH 4.2 (SVF) were controlled and followed non-fickian Release mechanism. Formulations containing IPC showed pH independent controlled Miconazole nitrate release without an initial burst release effect in both buccal and vaginal pH. Furthermore, F14 formulations showed satisfactory bioadhesive property and controlled release MN than all other formulations. However, the suitable combination of polymer with IPC exhibited the controlled release MN and satisfactory bioadhesive property with surface erosion along with swelling approached Zero-order release.**

INTRODUCTION

Candidiasis represents a major health challenge in immunocompromised patients like HIV and cancer reflecting in progressive immunodeficiency. In more than 90 % of HIV-positive patients, Candida infections are observed [1-3]. Among various types of candidiasis, oropharyngeal candidiasis and vaginal candidiasis are most prefound forms. Oropharyngeal candidiasis (OPC) is most prevalent in infants, the elderly, and compromised hosts and occurs in association with serious underlying conditions including diabetes, leukemia, neoplasia, steroid use, antimicrobial therapy, radiation therapy, and HIV infection [4,5]. One group of investigators reported that 28 % of cancer patients not receiving antifungal prophylaxis developed OPC and another group observed OPC in 57 % of immunocompromised patients [6]. Patients at greatest risk of developing OPC include those receiving corticosteroids and with prolonged neutropenia who are colonized with a *Candida* species [7]. Approximately 80-90 % of patients with HIV infection will develop OPC at some stage of their disease. Low numbers of organisms are the result of effective antifungal host defense mechanisms in the oral cavity. Low salivary flow rates correlate with higher prevalence rate of *Candida*. Genotyping of *Candida* strains obtained from HIV-positive patients with OPC and esophageal candidiasis compared to isolates from healthy individuals indicate an identical distribution frequency, suggesting that HIV associated candidiasis is not caused by unique or particularly virulent strains, but from defects in host defenses [8].

Candida vaginitis is the second most common vaginal infection. During the childbearing

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years, 75 % of women experience at least one episode of vulvovaginal candidiasis (VVC), and 40-50 % of these women experience a second attack [9]. Factors that enhance or facilitate germination (*e.g.,* estrogen therapy, pregnancy) tend to precipitate symptomatic vaginitis whereas measures that inhibit germination *(e.g.,* bacterial flora) may prevent acute vaginitis in women who are asymptomatic carriers of *Candida* [10].

The World Health Organization (WHO) Global Project on AIDS (GPA) recommended 100,000 IU of Nystatin orally 3 times daily for 7 days or gentian violet as a first-line treatment for Oropharyngeal candidiasis [11,12] .In further revisions to the WHO essential drug list, fluconazole is listed as a model "azole" compound, with various drugs serving as alternatives.

Other topical therapies for the treatment of oropharyngeal candidiasis include miconazole (in the form of buccal gels or tablets), clotrimazole (in the form of lozenges or troches), and amphotericin B (in the form of lozenges or rinses). The treatment regimen for all topical forms is repeated administration (*i.e.,* 3-5 times per day). The requirement for multiple applications is related to the relatively short intraoral exposure to topical antifungal agents as a result of rapid drug clearance via salivary production. The intraoral pharmacokinetics was documented for a miconazole-containing buccal gel, which provided maximal miconazole salivary concentrations immediately after application, followed by rapid clearance from the oral cavity [13,14].

Miconazole nitrate (MN) is a broad-spectrum antifungal agent that has been extensively applied for the management of dermal, buccal and vaginal candidiasis [15,16]. Several buccal drug delivery devices containing miconazole were developed such as chewing gum, oral gel, bioadhesive buccal tablets and buccal patches.

The main aim in the present study was to develop a novel mucoadhesive bucco-vaginal tablet to ensure satisfactory miconazole level in both buccal and vaginal cavity for prolonged periods. Literature review doesn't explore the effort for combined buccal and vaginal drug delivery system for candidiasis.

MATERIAL AND METHOD

Material

Miconazole nitrate obtained from Mayer's Health Care PVT Ltd., Bangalore, India. carbopol® 71G (Arihantt Trading Co., Mumbai, India). chitosan (Marine chemical, Cochin, India). Microcrystalline cellulose and talc were from Zydus Cadila, India. All other chemicals and reagents used were of analytical grade.

Preparation of carbopol®71G/chitosan complex

A carbopol® 71G aqueous solution (1 mg/mL) and chitosan acetic-aqueous solution (5 mg/mL) were mixed. The resulting precipitate (carbopol71G-chitosan IPC) was washed with distilled water and dried in freeze dryer for 24-h period. The powder was passed through a 200 µm sieve and used for further study.

Fourier transform infrared (FT-IR) spectroscopy study

The infrared absorption spectra of carbopol 71G, chitosan and their IPC were analyzed using a FT-IR spectrophotometer (8400S, Shimadzu, Japan). The pellets were prepared by pressing the sample with potassium bromide.

Differential scanning calorimetry (DSC)

Thermal analysis was carried out using a differential scanning calorimeter (DSC 50, Shimadzu Scientific Instruments, USA) for carbopol® 71G, chitosan and IPC. The samples were placed in an aluminum-sealed pan and preheated to 200 °C. The sample was cooled to room temperature and then reheated from 40 to 450 °C at a scanning rate of 10 °C/min.

Preparation of mucoadhesive matrix tablet

Mucoadhesive tablets were fabricated by direct compression method as shown in Table 1. The accurate quantity of miconazole and excipients were weighed. They were passed through sieve and thoroughly mixed using mortar and Pestle. The blend was lubricated and then compressed into compacts by the direct compression method using 8-mm flat-faced punches in KBr press (Technosearch, Mumbai, India) at 1 ton pressure with a dwell time of 1s.

Pharmaceutical properties of the mucoadhesive tablets

All the formulations were evaluated for uniformity of weight, and drug content as per pharmacopoeial method. The average weight was obtained for at least 20 units. The miconazole quantification was analyzed at 272 nm by UV spectrophotometer (UV-1700 Shimadzu, Japan). The thickness was measured using Mitotoyo screw gauge (Mitotoyo, Japan). Hardness was determined for at least 10 tablets using Erweka hardness tester (Erweka, India) and friability

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
chitosan	50	100	–	–	–	–	–	–	–	–	20	25	10	15
carbopol 71G	–	–	50	100	–	–	–	–	20	25	–	–	10	15
chitosan carbopol 71G physical mixture (1:1)	–	–	–	–	50	100	–	–	–	–	–	–	–	–
Interpolymer complex(IPC)	–	–	–	–	–	–	70	90	70	65	70	65	70	60
Microcrystalline cellulose	45	–	45	–	45	–	25	5	5	5	5	5	5	5
Talc	5	–	5	–	5	–	5	5	5	5	5	5	5	5

Table 1. Formulation chart. Miconazole nitrate = 50 mg. Total weight = 150 mg.

was evaluated for a sample of 20 tablets using Electrolab EF-2 Friabilator (Electrolab, India).

In vitro release of mucoadhesive tablets

The drug release rate from buccal compacts was studied using the orbital shaking incubator using (Remi CIS 24, India) 30 mL of phosphate buffer pH 6.8. The temperature was maintained at 37 ± 0.5 °C and 50 rpm. For every 1 h 3 mL sample was withdrawn, filtered through a millipore filter of 0.45 µm pore size and assayed spectrophotometrically at 272 nm. Immediately after each sample withdrawal, a similar volume of phosphate buffer pH 6.8 was added to the dissolution medium. Similarly in 500 mL of simulated vaginal fluid pH 4.2 in type II dissolution apparatus.

Bioadhesive strength

The force required to detach the bioadhesive tablets from the mucosal surface was applied as a measure of the bioadhesive performance. The method of Parodi *et al.*. [17] was slightly modified for measuring the bioadhesion strength of the tablets. The instrument is broadly composed of a modified two arm physical balance in which the right pan had been replaced by a formulation holding glass plate and counter balanced by a water collecting pan suspended to the left arm. The pan received a siphon tube from a 10 L bottle, always remaining above the water collecting pan. Nylon thread was used to suspend both the glass plate and the pan. An acrylate tissue mounting stage was attached to the center of a glass beaker. Glass beaker was filled with phosphate buffer (pH 6.8) and a magnetic stirrer provided with temperature control was used to maintain the temperature of phosphate buffer (pH 6.8) in glass dish at 37 ± 0.5 °C. A piece of sheep mucosa was tightly secured on the upper surface of the acrylate tissue mounting stage with thread. Tablets were fixed on the centre of the formulation holding glass plate with an adhesive (Fevi Quick®). The exposed tablet surface was moistened with phosphate buffer (pH 6.8) and left for 30 s for initial hydration and swelling. Then glass plate (with the film) was kept on the mucosal tissue secured on the tissue mounting stage in such a way that tablet completely remained in contact with mucosa. The glass plate (weight 50 g) itself acted as a preload. After the preload time, water collecting pan was suspended to the left arm and water was added in it, by the siphon tube, at a constant rate of 200 drops per minute until detachment of the film from mucosal surface took place. A support was kept under the water collecting pan to hold it at the time of detachment. Weight of water collected in the pan at the time of detachment was measured. The experiment was performed in triplicate

Swelling studies

The swelling index of the prepared mucoadhesive miconazole tablets was determined by weighing five tablets and recording their weights before placing them separately in weighed beakers. The total weight was recorded (*W1*). Four milliliters of phosphate buffer pH 6.8 (similarly with simulated vaginal fluid pH 4.2) was added to each beaker and then placed in an incubator at 37 ±0.5 °C. At time intervals of 2, 4, 6 and 8 h excess water was carefully removed, and the swollen tablets were weighed (*W2*). The experiment was repeated three times, and the average W1 and W2 were reported.

The swelling index (*SI*) was determined from the Eq. [**1**]

$$SI = (W2\text{-}W1)/W1 \times 100 \quad [1]$$

Kinetic analysis

Drug release from simple swellable systems may be described by the power law expression and is defined by Eq. [**2**]:

$$M_t/M_\infty = K_1 t_n \quad [2]$$

where M_t is the amount of drug released at time *t*, M_∞ is the overall amount of drug released, K_1 is the release constant; n is the release or diffusional exponent and M_t/M_∞ is the cumulative drug concentration released at time *t*.

The release exponent (*n*) value was used for interpretation of the release mechanism from the compacts. The dissolution data were modeled by using PCP disso v2.01 (Bharathi Vidhyapeeth, Deemed University, Pune, Maharashtra, India).

RESULTS AND DISCUSSION

In our previous paper, carbopol® 71G polymer and the influence of formulation expedients were evaluated on buccal bioadhesive tablet of fluconazole [18]. carbopol® 71G is a well known bioadhesive polymer for buccal drug delivery and it's well suitable for buccal pH. However the major candidiasis occur in buccal and vaginal cavity, it could be important to achieve pH independent miconazole release pattern to exert drug action in both candidiasis site. carbopol® 71G doesn't able to show the pH independent drug release action and was complexed with chitosan, a cationic biodegradable natural polymer. The main benefit of this novel bucco-vaginal drug delivery system is their pH independent drug release pattern in both buccal and vaginal region due to the interpolymer complex of chitosan and carbopol® 71G. Furthermore, bucco-vaginal drug delivery could provide prolonged effect of miconazole and better adhesiveness on buccal and vaginal tissue in comparison to conventional drug delivery systems. Multipurpose use, dose reduction and dose dependent side effects could be another and essential benefit of novel drug delivery system.

Characterization of chitosan-carbopol 71G IPC

Figure 1 shows the IR spectra of chitosan, carbopol® 71G and the carbopol/chitosan IPC in 1000-2000 cm^{-1} and 1400-1800 cm^{-1} .The interaction between chitosan and carbopol® has been studied by several investigators [19,20]. The studies indicated that interpolymer complex could be formed by the electrostatic interaction between the COO^- group of carbopol® 71G and NH_3^+ group of chitosan. The protonation of chitosan and dissociation of carbopol solution was successfully accomplished by solution of chitosan and carbopol were dissolved in the acetic acid solution and water, respectively.

The possible groups involved in the formation of interpolymer complex are 2-aminoglucose unit of chitosan and carbonyl group of carboxylic acid in carbopol® 71G. The absorption bands of various groups and IPC was confirmed by IR spectra.

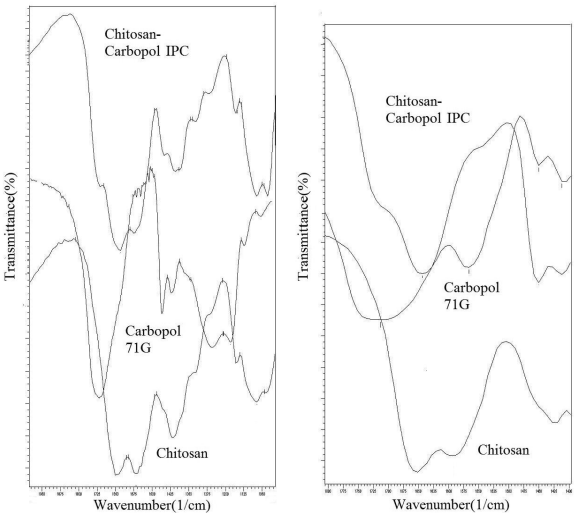

Figure 1. FT-IR Spectra of chitosan, carbopol 71G and IPC in 2000-1000 and 1800-1400 cm^{-1}.

The peak at 1715 cm^{-1} in the IR spectrum of carbopol® 71G was assigned to the carbonyl group of carboxylic acid. The amine group of the 2-aminoglucose unit and the carbonyl group of the 2-acetaminoglucose unit of chitosan showed absorption bands [21] at 1590 and 1656 cm^{-1}. The IR spectrum of the IPC showed that the peak of 1590 cm^{-1} assigned to the amine band of chitosan was shifted to 1640 cm^{-1}, indicating that the amine group was protonated to a NH_3^+ group in IPC [22]. The bands at 1565 and 1411 cm^{-1} were assigned to the symmetric and asymmetric stretching of the COO^- group [23].

In addition, the NH_3^+ band was known to appear 24 between 1600 and 1460 cm^{-1}. Moreover, the peak of NH_3^+ groups in the complex between chitosan and poly(acrylic acid) was known to appear [25] at 1520 cm^{-1}.

Figure 2 shows the DSC thermograms of chitosan, carbopol® 71G, and the carbopol/chitosan IPC. A broad endothermic peak appeared approximately at 90 °C may be attributed to bound water. An exothermic peak attributable to the decomposition of chitosan appeared at approximately 320 °C [26,27]. In the DSC thermogram of carbopol, the decomposition of carbopol was observed at approximately 280° C at which the carbopol had melted and decomposed sequentially [28,29]. The broad endothermic peak at 85 °C was attributed to the physically bound-water.

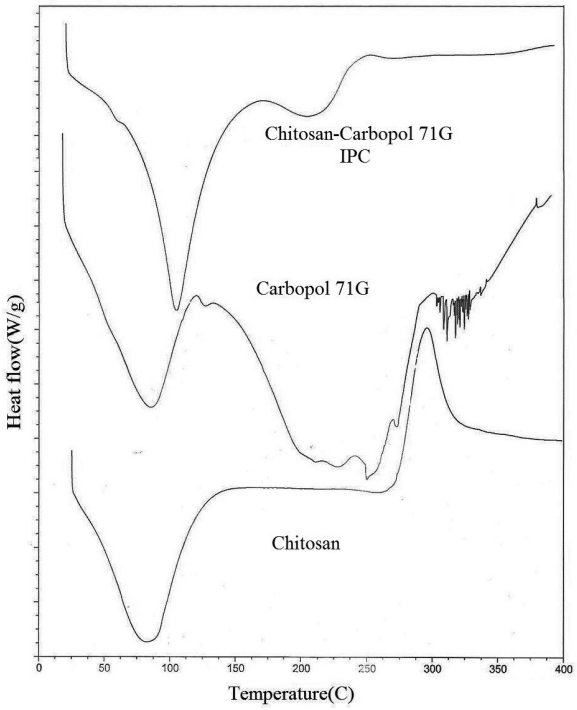

Figure 2. DSC thermogram of chitosan, carbopol 71G and chitosan-carbopol 71G IPC.

The broad endothermic peak of chitosan is attributed to amine group; hence the water absorption ability of chitosan depends on the account of its amine group. The sharp and small endothermic peak in IPC is due to bound water. The smaller endothermic peak than chitosan and carbopol were attributed to the reduction in the amine group due to complexation with carboxyl group and amine group. Therefore, the water absorption capacity of the IPC may be lower than chitosan. The reduced water absorption capacity might result in the slow disintegration of the IPC matrix and the extension of drug release from the IPC matrix

Physicomechanical properties of the tablets

As shown in Table 1, polymeric tablet formulations containing various ratios of chitosan, carbopol® 71G, chitosan-carbopol® 71G physical mixture and IPC loaded with 50 mg of MN, were prepared and their technological properties were examined. All the formulations passed test for weight variation, drug content (99.90-99.95 %) and % friability (0.41-0.58 %). The hardness of the buccal compacts ranged from 5.11 to 5.23 kg/cm2 and thickness ranged between 2.01-2.1 mm with 2 ton pressure. The values of variation in weight, drug content, and friability were found to be within the limits of conventional oral tablets stated in the *Indian Pharmacopoeia* [30].The IR spectra of pure MN and its physical mixture with carbopol® 71G, chitosan and chitosan/carbopol® 71G IPC did not show any significant differences (data non shown).

In vitro release of MN

One of the key steps in the formulation process of controlled drug delivery systems is the selection of the polymeric matrix formers, due to the polymeric gel layer usually acts like a rate-controlling membrane, resulting in linear release of the drug. Moreover, drug release patterns can be greatly either increased or decreased by increasing the amount of the polymers. Herein, drug release profiles of the polymeric compacts containing polymers formulated with microcrystalline cellulose (Figs. 3a, 3b, 4a, 4b) are described.

F1 and F2 formulations containing chitosan alone exhibits variation in rate of drug dissolution in buccal pH 6.8 and vaginal pH 4.2. In buccal pH 6.8 the rate of drug release is faster than the vaginal pH 4.2. The simulated vaginal

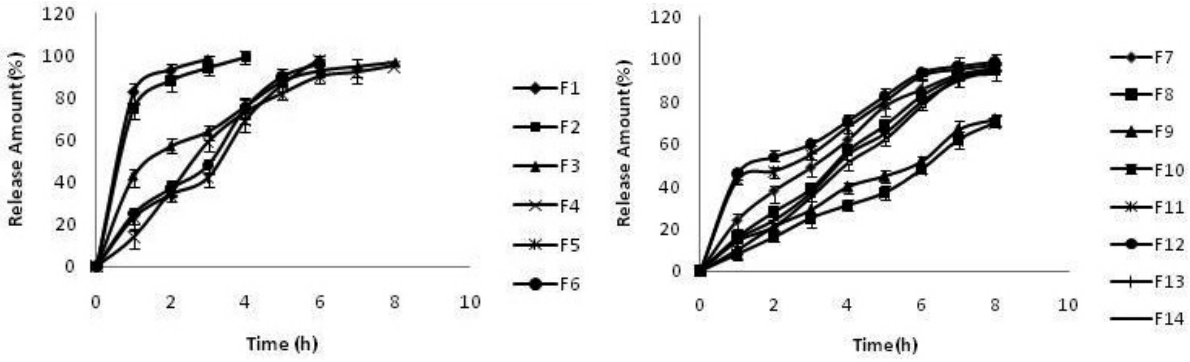

Figure 3. Release profile of miconazole from F1-F14 formulations in buccal at pH 6.8

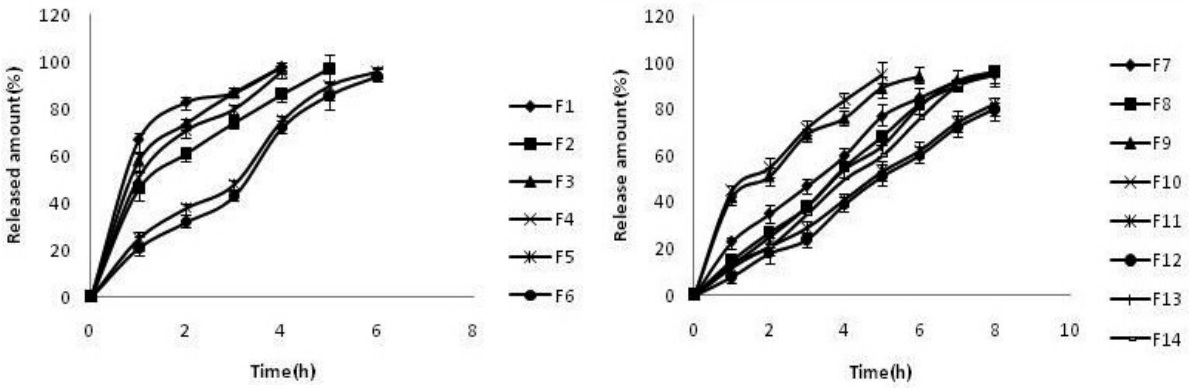

Figure 4. Release profile of miconazole from F1-F14 formulations in simulated vaginal fluid at pH 4.2.

fluid is little acidic than buccal pH, in acidic pH the chitosan has the ability to form gel layer around the compact, which retards the rate of drug release for the compacts. The faster drug release of chitosan in formulation F1 &F2 is due to the easy disintegration and poor gelling property near neutral pH [31].

The rate of drug dissolution was influenced by the pH of the dissolution medium for F3 and F4 formulations containing carbopol® 71G formulations. The presence of carboxyl groups will dissociate at pH 6. 8 result in the formation of a swollen gel hence the rate of drug release are alterated at Buccal pH 6.8. However the carboxyl groups of carbopol® 71G will not dissociate at simulated vaginal pH resulting in a less viscous gel around the matrix compact. Therefore the rate of drug dissolution from the carbopol® 71G compacts at buccal pH 6.8 was slower than that at vaginal pH [32].

The F4 formulation showed the controlled release pattern than the F3 formulation due to increased concentration of polymer. In buccal pH, with increase in the concentration of carbopol 71G the dissociation of more carboxyl group results in the formation of a swollen gel inturn the drug penetrates the gel and reaches the dissolution medium. The more the dissociation of the carboxyl group the more will the swollen gel hence results in the decreased drug release pattern.

However in the vaginal pH the more the concentration of carbopol 71G there will be no dissociation of carboxyl group hence results in the less viscous gel around the matrix compact. Therefore the rate of drug dissolution from the F4 is less than F3 formulation in vaginal pH.

F5 and F6 formulation containing the physical mixture of chitosan and carbopol® 71G exhibited the entire dose after 4 h only. This is due to the relatively faster erosion and complete disintegration of the compact prepared from the chitosan- carbopol® 71G powder mixture. The easy disintegration property of chitosan at neutral pH hindered the property of carbopol® 71G polymer like formation of swollen gel. This leads to the formation of larger pores in the compacts, Hence the desired drug release pattern was diminished at buccal pH. In vaginal pH also the carbopol® 71G properties overrules the property of chitosan property of forming gel in lower pH. Hence the ability to form the gel in lower pH of chitosan is diminished lacking in the desired drug release pattern. The dissociation of carboxyl group of carbopol® 71G in the formulations F3 & F4 results in the formation of a swollen gel but at the vaginal pH the dissociation of carboxyl groups is diminished which in turn doesn't produced the desired drug release pattern. The chitosan-carbopol® 71G IPC compacts showed the pH independent drug release pattern than the chitosan and carbopol® 71G compacts. The dissolution pattern of Miconazole nitrate from compacts at buccal and vaginal pH was almost similar. The increase in concentration of IPC in compacts decreases the drug release pattern. The IPC incorporated into the tablets forms the gel around the tablet and makes the Miconazole release in the slow manner.

The formed complex between the chitosan and carbopol involved in the preparation of tablet comes in contact with the dissolution medium uptake the dissolution medium and swells slowly and forms the gel layer around the compact. The Miconazole then penetrates the gel layer and reaches the medium. The gel layer form the barrier and acts as rate limiting step to the drug release. The increase in the concentration of IPC retards the release pattern of Miconazole.

The other formulations like F11-F14 containing mixture of IPC, chitosan and/ Or carbopol were subjected for further studies. The proper proportion of chitosan carbopol and IPC exhibited the similar drug profile as that of only IPC containing formulations.

Mucoadhesion study

The formulations F7-F14 was considered for the mucoadhesive studies based on the desired drug release studies. Figure 5 indicates bioadhesive strength (with sheep mucosa). It is known that polymer concentration exhibit a significantly influence the strength of mucoadhesion [33].

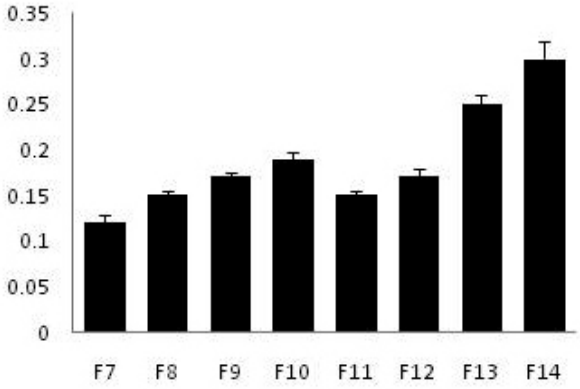

Figure 5. Mucoadhesive strength of the formulation.

Formulations F7 and F8 exhibits the least bioadhesive strength. The formed complex lacks the functional group which is involved in the complex formation; hence the satisfactory bioadhesive strength was not evolved. The formed complex lacks the strong binding but finds little binding to the mucosal surfaces via hydrogen bonding interactions [34]. F9 and F10 formulations showed little higher bioadhesion than F7 & F8. This may be due to the chitosan involvement in the formulations. The increase in concentration of chitosan also increases the bioadhesive property.

The formulations containing carbopol® 71G formulations exhibits much more bioadhesive strength than above mentioned formulations may be due to carboxylic group of carbopol® 71G forms tight bond with mucous proteins. F14 formulation exhibits the highest bioadhesion than all other formulations, may be due to combination of chitosan and carbopol® 71G posses the synergistic effect of bioadhesion.

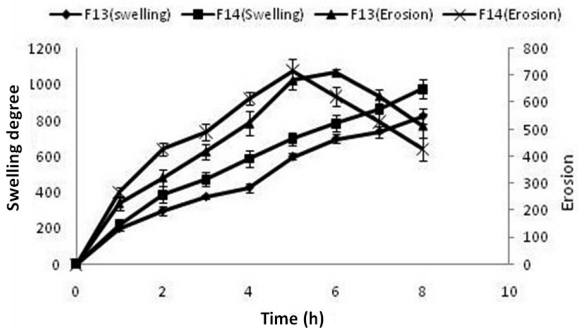

Figure 6. Swelling studies and erosion studies of F13 and F14 formulations.

Swelling studies of tablets

The formulations which exhibited the desired drug release profile and mucoadhesive studies are considered for the water uptake studies of compacts. In buccal pH 6.8 the surface of the compacts gradually swelled with the core remained intact whereas at vaginal pH the compacts become soft and swelled quickly.

The erosion of compacts at buccal pH 6.8 was not significant and whereas in vaginal pH 4.2 the compacts has eroded for F13 & F14 Formulations. The presence of chitosan and carbopol along with IPC affects the swelling behavior. F13 & F14 exhibited the optimum swelling in both buccal and vaginal pH. Whereas in F13 and F14 shows erosion in vaginal pH, which may be due to the carbopol polymer which doesn't dissociate in the acidic pH . Hence erosion takes place in vaginal pH 4.2 (Fig. 6).

Kinetic analysis

According to the drug release kinetics it is clear that the matrix type tablets prepared from the IPC approached zero-order release for MN. F14 formulation exhibits the controlled release profile with zero-order release (R = 0.9974; K = 12.2954) in vaginal pH 4.2, which might be due to rapid swelling and erosion. Whereas in buccal pH 6.8, apparent first-order drug release profile (R = 0.9945; K = 10.283) this might be due to slow swelling and no erosion of polymers.

CONCLUSIONS

The chitosan-carbopol® 71G interpolymer complex demonstrated the high potential for both bucco-vaginal drug delivery systems for the controlled release of miconazole nitrate in candidiasis. F14 formulation is the optimized formulation. The IPC reduces the pH dependent release profile of carbopol® 71G and exhibits

the pH independent drug release pattern without initial burst release effect that approaches zero-order kinetics. Suitable combination of chitosan, carbopol® 71G and IPC exhibit very good swelling, bioadhesive property and pH independent release of MN.

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